A pneumatic valve controlled microdevice for bioanalysis

Xiaohu Zhou, Xuechang Zhou, and Bo Zheng[a)]
Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

(Received 25 August 2013; accepted 7 October 2013; published online 21 October 2013)

This paper describes a pneumatic valve controlled microdevice for performing mixing and reaction. This microdevice combined the degassed polydimethylsiloxane (PDMS) pumping method with a syringe-actuated valve system to control the dispensing and mixing of nanoliter solutions. The syringe was used to manually generate vacuum and to open the valves. Upon the opening of the valve, the microchamber was filled with the solution, which was driven by the external atmosphere through the degassed PDMS microchannel. With this microdevice, the enzymatic kinetics of alkaline phosphatase converting the fluorescein diphosphate was studied, and the Michaelis-Menten kinetics was analyzed. The microdevice has the advantages of simplicity and low cost in fabrication and operation. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4826158]

I. INTRODUCTION

To automate serial assays and reduce the reagent consumption, flow injection analysis was developed in the 1970s as a general solution-handling and data-collection technique.[1,2] Lately, many microfluidic platforms emerged to further improve the solution-handling and reduce reagent consumption in chemical analysis. Typical microfluidic platforms for analysis include hydrodynamic focusing,[3,4] droplet,[5,6] digital microfluidics,[7,8] microreactor array,[9–12] etc. Both hydrodynamic focusing and droplet methods are capable of rapid mixing within μs to ms and therefore are appropriate for analyzing rapid kinetics. However, the reagent consumption in both the hydrodynamic focusing and droplet methods is relatively large due to the fast continuous flow during the analysis. The digital microfluidics and microreactor array platforms have the advantage of smaller reagent consumption, although the mixing time is usually in seconds.[7–11] In digital microfluidics, the fabrication of the microchip coupled with electrodes is complex and the hydrophobic electrode surface required by electrowetting is prone to the protein deposition,[8] which limit the application of digital microfluidics. The microreactor array platforms are simple to fabricate. However, the microwell array-based platform relies on manual operation including alignment, leaving room for improvement in automation and operation consistency.

To precisely control the fluidic flow, the on-chip pneumatically activated valve is developed.[13] The fabrication of the pneumatically activated valves is based on soft-lithography procedure.[14,15] With the advantages of simple fabrication, easy integration and fast response time, the pneumatic valve systems are more widely used[9,16–20] than many other types of valves for microfluidics.[21–29] However, the pneumatic valve also has the limitation that expensive external hardware such as gas cylinders with pressure regulators is required. To address this issue, the screw-actuated valves[30,31] have been developed to directly[30] or indirectly[31] control the fluid flow with small machine screws. However, these valve systems are slow in response time and difficult to be integrated. Herein we developed a different type of manually actuated pneumatic valves based on latching pneumatic valves.[25,32] The valves allow us to combine with the degassed polydimethylsiloxane (PDMS) dispensing method[10,33–35] to build a microdevice for general serial assays.

[a)]Author to whom correspondence should be addressed. Electronic mail: bozheng@cuhk.edu.hk. Telephone: 852-3943-6261. Fax: 852-2603-5057.

In the current work, manually actuated pneumatic valves were integrated in the microdevice, which circumvented the need for the expensive electromechanically operated valves or syringe pumps.[32] We combined the degassed PDMS pumping method[10,33–35] with the manually actuated pneumatic valves to control the solution dispensing and mixing. To illustrate the application of the device, we used the microdevice to study the enzymatic kinetics of alkaline phosphatase. The microdevice consumes similar amount of reagent as the digital microfluidics does but is much simpler to fabricate and operate.

II. METHOD

A. Design of the pneumatic valve controlled microdevice

The schematic illustration of the pneumatic valve controlled microdevice is shown in Figure 1. There are three reagents to be dispensed into the microwells: enzyme solution, substrate solution, and the buffer solution for dilution (Figure 1). As shown in Figure 1(b), PDMS is first degassed in a vacuum chamber to remove the air in PDMS. Once the PDMS microdevice is taken out and exposed to atmosphere, air will slowly diffuse back to the PDMS both through the surface and the PDMS microchannel. When the reagent is placed at the inlet by

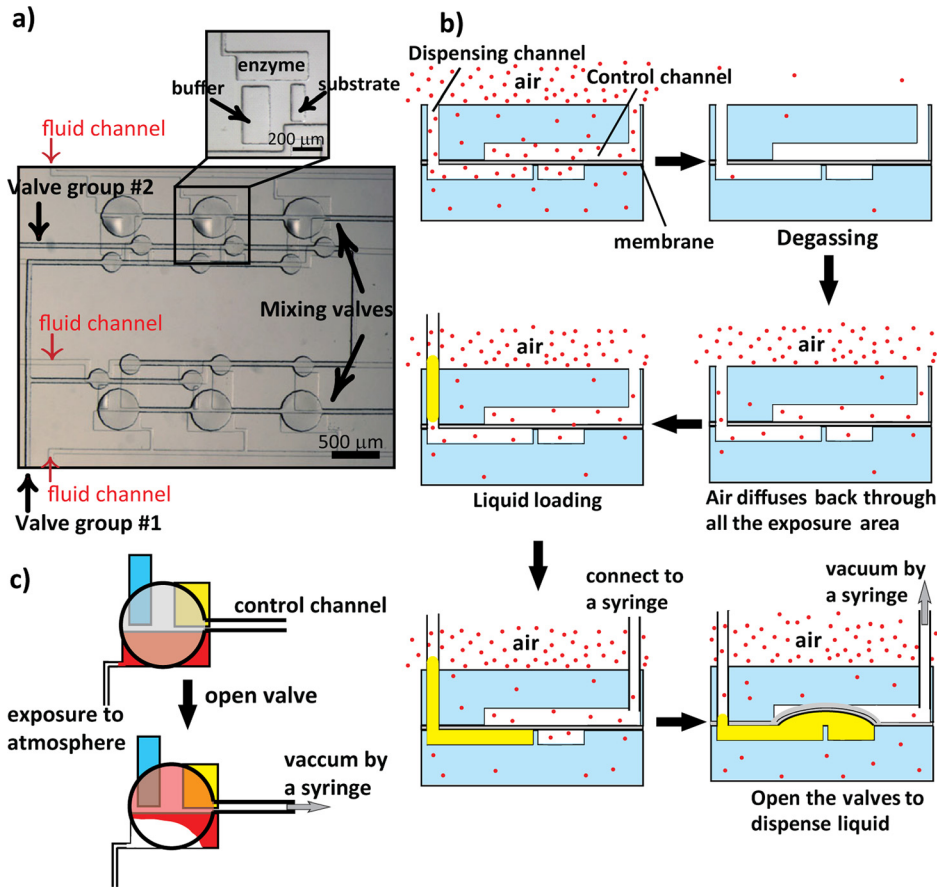

FIG. 1. (a) The microphotograph of three parallel dispensing and mixing units in the pneumatic-valve controlled microdevice. On the microwell layer, there are three types of microwells for enzyme, substrate, and buffer solutions, respectively. On the valve layer, there are one group of valves for mixing and two groups of dispensing valves: Valve group #1 for buffer dispensing and Valve group #2 for substrate solution dispensing; (b) The dispensing strategy by degassed PDMS and the valve. Upon opening the dispensing valves, the external atmosphere will drive the liquid reagents into the microchannel due to the internal vacuum in the microchamber generated by the degassed PDMS. The red dots represent gas molecules in air. (c) The valve-controlled mixing: (Top view) Upon opening the mixing valves, the external atmosphere will drive the liquid reagents in the enzyme microwells to mix with other liquid reagents over the boundaries between the microwells.

either inserting pre-loaded tubing or depositing the target solution directly at the inlet, the air flow into the microchannel is blocked. The internal pressure in the microchannel will decrease due to the continuous dissolving of air inside the microchannel into the PDMS. As a result, the pressure difference between the external atmosphere and the PDMS microchannel will drive the reagent into the microchannel.[10]

In the experiment of enzyme analysis, the enzyme solution was dispensed into the microwells directly through the fluid channels (Figure 1(a)). The substrate or buffer solution was first aspirated into the microchannels, and then the pneumatic valves will control the dispensing of the substrate or buffer solution from the microchannels to microwells (Figure 1(b)). After the dispensing completed, enzyme assays was carried out by opening the mixing valves (Figure 1(c)).

The dispensing of the sample solution was critically dependent on the vacuum power of the degassed PDMS,[10,33] which in turn depended on the thickness of the PDMS slabs and the degassing time. In the current design, both the control layer and the fluid layer were ~5 mm thick. The experiment was immediately performed when the microdevice was taken out from the ~5 kPa vacuum chamber after 30 min degassing. The sample solution could completely fill the microchannel in 10 s.

B. Fabrication of the microfluidic microdevices

The pneumatic valve controlled microdevice was fabricated by soft lithography.[14,15] Two molds were first prepared by photolithography: one for the pneumatic valve control layer and the other for the reaction layer. A third flat silicon wafer was used to make the intermediate thin PDMS membrane. Mixture of PDMS precursor and curing reagent (Sylgard 184, Dow Corning) was used to fabricate the PDMS replica from the molds following the previously reported procedure.[13,17,18]

To assemble the microdevice, the PDMS replica layer containing the pneumatic valves' components was irreversibly bound with the thin membrane (thickness ~ 10 μm), and then reversibly bound with the PDMS replica layer containing the reaction microwells (Figure 2). The pneumatic valve control layer contained the main channels (width 50 μm × height 50 μm) and the valve chambers (diameter 500 μm × height 50 μm for mixing; diameter 200 μm × height 50 μm for dispensing), while the reaction layer contained the main channels (width 50 μm × height 50 μm), branch channels (width 25 μm × height 50 μm) and microwells. The volume of the microwells for the enzyme reagent was constant at 5 nl (length 500 μm × width 200 μm × height 50 μm), while the volume of the substrate microwells varied from 5 nl to 0.625 nl, and the total volume of substrate and buffer in each reaction unit was also constant at 5 nl.

C. Demonstration of the pneumatic valve controlled microdevice

The reagents loading and mixing of the pneumatic valve controlled microdevice is demonstrated in Figures 3 and 4. As described previously, after the degassed PDMS microchip was taken out from the vacuum chamber, the enzyme solution was aspirated into the microwells directly through the branch channels. At the same time, the buffer solution was aspirated into the center main channels, and the dispensing valves for the buffer were opened to dispense the buffer into the buffer microwells (Figure 3). After the buffer dispensing completed, the buffer dispensing valves were closed, and the solution in the main channels would be pushed into the chamber at the end. When air segments appeared in the main channels, the substrate solution started to be aspirated into the main channels, and the substrate dispensing valves would be opened. After all the microwells were filled with the proper solutions, and there was no solution in the channels, the large mixing valves would be opened to start the reaction (Figure 4). The valves were robust and fully closed and opened repeatedly throughout the experiment.

For the current design of six reaction units, 1 ml plastic syringe is enough to control the dispensing and mixing. We believe that with larger syringe up to a hundred reaction units

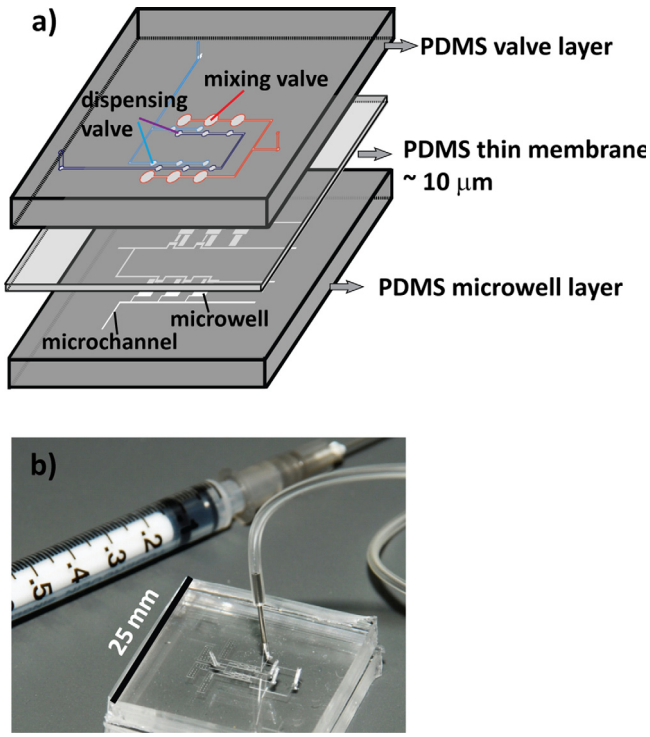

FIG. 2. The microdispensing system with pneumatic valves is a multiplayer PDMS microdevice: (a) The configuration of the multiplayer PDMS microdevice. The microwell layer shows only the reaction area in the scheme. (b) The photograph of the syringe-controlled valve and the dispensing system.

can be integrated. The number of the reaction units in the device is limited by the vacuum power of degassed PDMS. We have previously reported that with a single dispensing channel the filling time increased with the number of microchamber. Up to 100 microchambers, the dispensing time was about 30 min.[10] To integrate even more reaction units in one

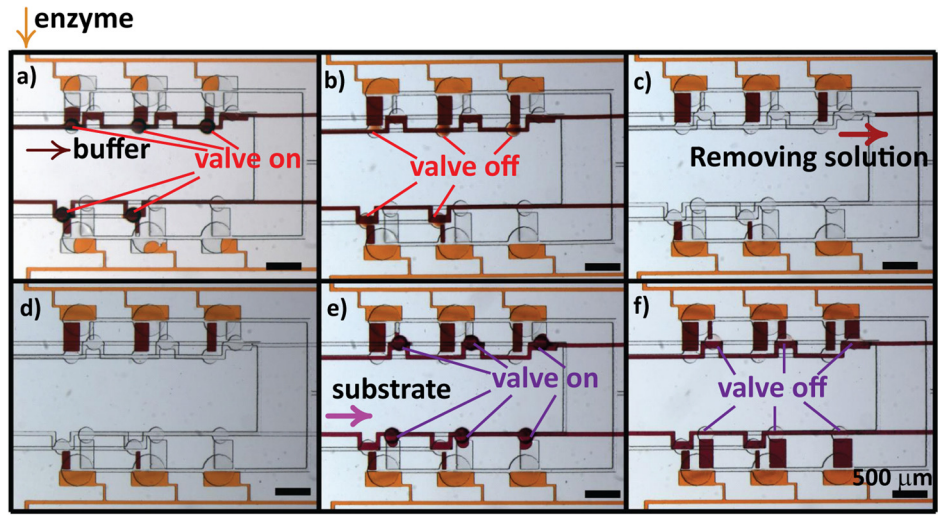

FIG. 3. Demonstration of the pneumatic valves controlled solutions dispensing. The device contains six reaction units, each of which has three microwells of different volume ratios for serial analysis. (a) and (b) Enzyme solution was aspirated to the microwells directly through the branch channels. At the same time, the dispensing valves for buffer were activated to dispense the buffer solution. (c) and (d) After the buffer solution was dispensed completely, the buffer solution in channel was removed (e) and (f) the third solution of substrate was dispensed by opening the substrate dispensing valves. For better illustration, dye molecules were added into the three solutions: $[Fe(phen)_3]^{2+}$ for enzyme, $[Fe(SCN)_x]^{3-x}$ for buffer and $KMnO_4$ for substrate solutions.

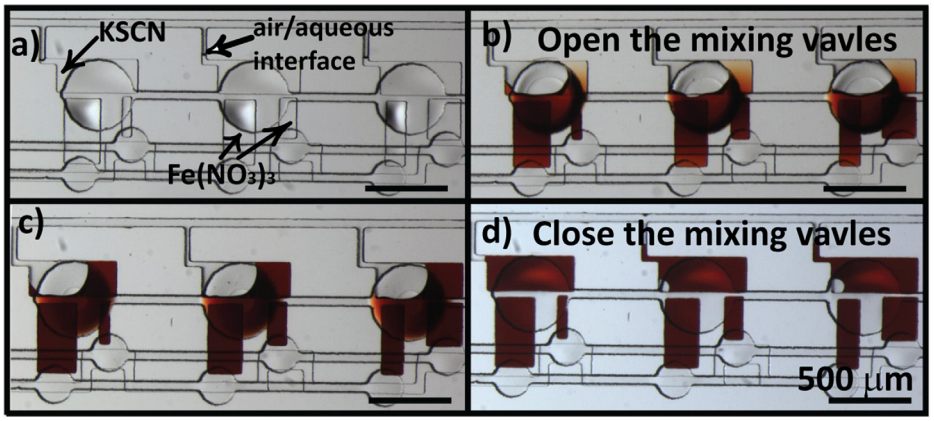

FIG. 4. Demonstration of the pneumatic valves controlled mixing. After all the microwells were filled with the proper solutions, and there was no solution in the channels, the large mixing valves were opened to initiate the reaction. The solution turned dark red when $Fe(NO_3)_3$ mixed with KSCN.

microdevice, the parallel dispensing microchannels and external degassed PDMS pump are needed.[33,35]

III. RESULTS AND DISCUSSIONS

A. Reagents and materials

Alkaline phosphatase (Sigma-Aldrich) was dissolved in 10 mM diethanolamine (DEA) buffer, pH 10.1, containing 1 mM $MgCl_2$. Enzyme concentrations were 6 U/ml for the standard curve experiments and 0.1 U/ml for kinetic analysis. Fluorescein diphosphate (Sigma-Aldrich) solutions of various concentrations were prepared in 10 mM DEA buffer.

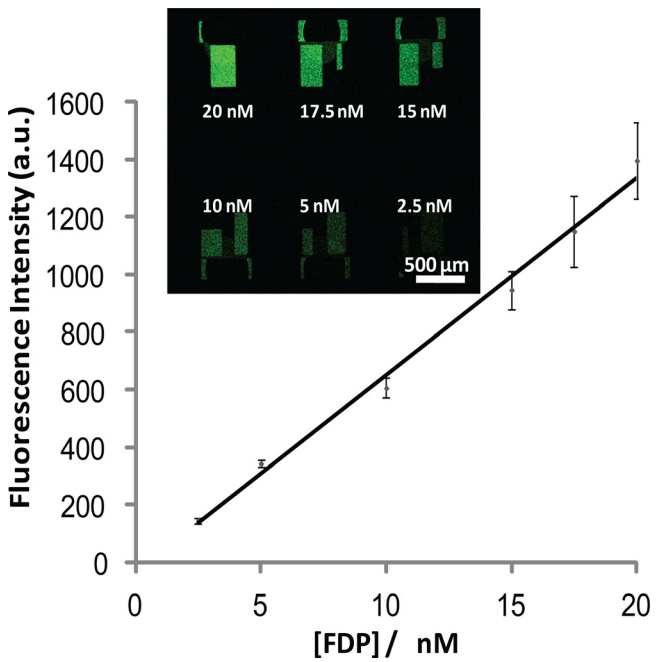

FIG. 5. Results of the FDP calibration experiment. The straight line is the linear fitting of the data points. The error in the data points ranged from 3% to ~10% RSD. The inset shows the fluorescent images of the reaction units containing different FDP concentrations.

The fluorescent images were taken by the confocal laser scanning microscope (Eclipse C1si, Nikon). The fluorescent intensities were analyzed by the NIS-Elements (Nikon).

B. Enzymatic activity

For this pneumatic valve controlled microdevice, one chip consisted of six reaction units, while each reaction unit contained three microwells to accommodate the enzyme, substrate and buffer solutions, respectively. The enzymatic reactions started immediately after opening the mixing valves to mix the reagents. With the constant total volume and the different volume ratios of the substrate and the buffer solutions, we were able to get the kinetics data with six different substrate concentrations at the same enzyme concentration in one chip.

Calibration curve was first generated by this microdevice. The enzyme concentration was 6 U/ml, and the substrate concentrations for the valve-based system were 20, 17.5, 15, 10, 5, 2.5 nM (Figure 5). The fluorescence response was linear over a range of two orders magnitude of substrate concentration, which was limited by the detector.

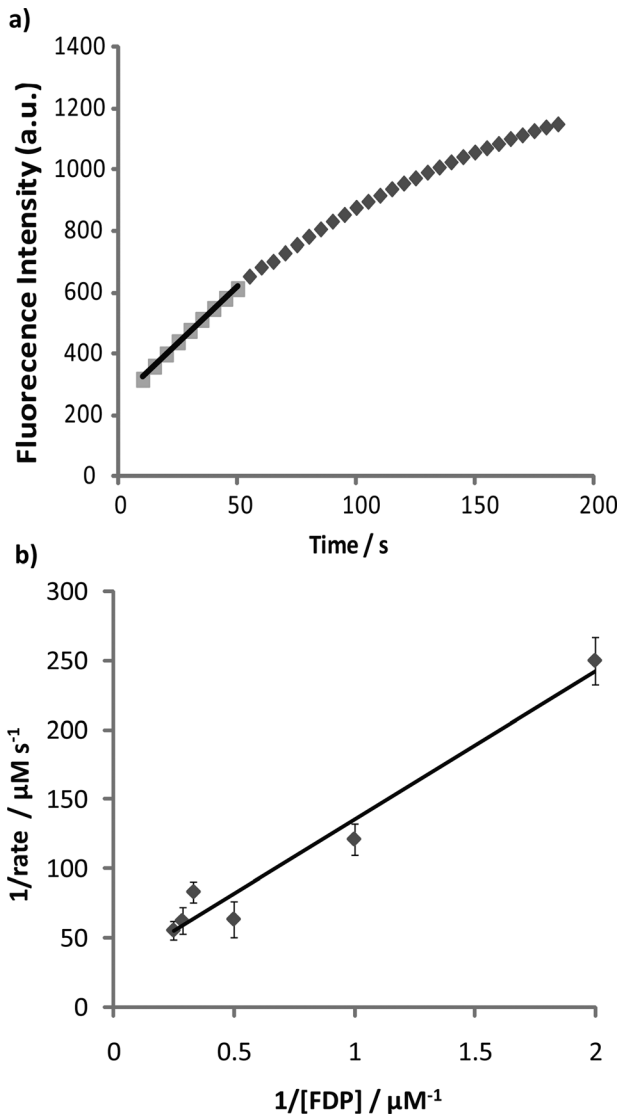

FIG. 6. (a) Plot of the fluorescence signals against time in the 96-well microplate after alkaline phosphatase (0.1 U/ml) and FDP (1 μM) was mixed. (b) Lineweaver-Burk plot of the initial reaction velocity versus $[FDP]^{-1}$ with microdevice. The values of V_{max}, K_m, and k_{cat} were obtained by analyzing the plots, $V_{max} = 28$ nM s^{-1}, $K_m = 2.9$ μM, and $k_{cat} = 156$ s^{-1}.

C. Enzymatic kinetics

About 30 s was required to mix the reagents completely with the microdevice, which was the dead time for studying the kinetics. Therefore, the experimental conditions should be optimized so that the reaction rate remained unchanged in the initial 50 s, considering that the data collected in the first 20 s (from 30 s to 50 s) was used to calculate the initial rate. As shown in Figure 6(a), the experiment results done in 96-well microplate showed that during the first 50 s the reaction rate remained unchanged, so 0.1 U/ml or lower concentration of alkaline phosphatase was used to study the kinetics.

Mixing in the microchambers relied on both the diffusion and convection. The convection was generated by the quickly open the mixing valves. For the current study, mixing could be completed within 30 s. The boundary width between chambers and the height of the control level affected the mixing efficiency. The boundary width was related to the diffusion distance of the reagents. Therefore, the wider boundary width led to longer mixing time and lower mixing efficiency. On the other hand, the higher the control level was, the more liquid was driven through the opening of the valve, which would enhance the mixing efficiency. The microchamber can be as small as $50\,\mu\text{m} \times 50\,\mu\text{m}$. Large microchamber size on the scale of mm would be inappropriate for kinetics studies as the mixing time would be too long, unless extra components such as piezoelectric actuator are integrated to enhance the mixing.[36,37]

The initial reaction velocities were calculated and were plotted against fluorescein diphosphate (FDP) concentrations in a double-reciprocal Lineweaver-Burk plot [Figure 6(b)]. The value of K_m and k_{cat} were obtained by analyzing the plots. The k_{cat} value was $156\,\text{s}^{-1}$, which was consistent with the reported values around $\sim 100\,\text{s}^{-1}$.[8,38]

IV. CONCLUSIONS

In this work, we demonstrated that the pneumatic valve controlled microdevice could achieve solution dispensing and mixing by a single syringe. By pulling the syringe, a vacuum was generated at the control channel, causing the deformation of the PDMS membrane. Due to the pre-stored internal vacuum power generated by the degassed PDMS, the external atmosphere drove the fluids into the microchambers.

The microdevice allowed on-chip dilution and prepared different concentrations of the analytes in one run. The microdevice was validated by performing the Michaelis-Menten kinetics analysis of alkaline phosphatase. The microdevice is simple, inexpensive and easy to operate, and is suitable for serial assays and screening[10,39] in bioanalysis.

ACKNOWLEDGMENTS

This work is supported by the Research Grants Council of Hong Kong (404212).

[1]J. Ruzicka and E. H. Hansen, *Flow Injection Analysis* (Wiley-Interscience, 1988).
[2]J. Ruzicka and E. H. Hansen, Trend Anal. Chem. **27**, 390 (2008).
[3]J. B. Knight, A. Vishwanath, J. P. Brody, and R. H. Austin, Phys. Rev. Lett. **80**, 3863 (1998).
[4]L. Pollack, M. W. Tate, N. C. Darnton, J. B. Knight, S. M. Gruner, W. A. Eaton, and R. H. Austin, Proc. Natl. Acad. Sci. U.S.A. **96**, 10115 (1999).
[5]H. Song and R. F. Ismagilov, J. Am. Chem. Soc. **125**, 14613 (2003).
[6]A. Huebner, L. F. Olguin, D. Bratton, G. Whyte, W. T. S. Huck, A. J. de Mello, J. B. Edel, C. Abell, and F. Hollfelder, Anal. Chem. **80**, 3890 (2008).
[7]T. Taniguchi, T. Torii, and T. Higuchi, Lab Chip **2**, 19 (2002).
[8]E. M. Miller and A. R. Wheeler, Anal. Chem. **80**, 1614 (2008).
[9]C. L. Hansen, E. Skordalakes, J. M. Berger, and S. R. Quake, Proc. Natl. Acad. Sci. U.S.A. **99**, 16531 (2002).
[10]X. Zhou, L. Lau, W. W. L. Lam, S. W. N. Au, and B. Zheng, Anal. Chem. **79**, 4924 (2007).
[11]W. B. Du, L. Li, K. P. Nichols, and R. F. Ismagilov, Lab Chip **9**, 2286 (2009).
[12]H. M. Hegab, A. ElMekawy, and T. Stakenborg, Biomicrofluidics **7**, 021502 (2013).
[13]M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, and S. R. Quake, Science **288**, 113 (2000).
[14]J. C. McDonald and G. M. Whitesides, Acc. Chem. Res. **35**, 491 (2002).
[15]D. C. Duffy, J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides, Anal. Chem. **70**, 4974 (1998).
[16]J. W. Hong and S. R. Quake, Nat. Biotechnol. **21**, 1179 (2003).
[17]Z. Y. Han, W. T. Li, Y. Y. Huang, and B. Zheng, Anal. Chem. **81**, 5840 (2009).
[18]Z. Y. Han, Y. Y. Chang, S. W. N. Au, and B. Zheng, Chem. Commun. **48**, 1601 (2012).

[19]H. K. Wu, A. Wheeler, and R. N. Zare, Proc. Natl. Acad. Sci. U.S.A. **101**, 12809 (2004).
[20]Y. H. Liu, C. H. Wang, J. J. Wu, and G. B. Lee, Biomicrofluidics **6**, 034119 (2012).
[21]D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss, and B. H. Jo, Nature **404**, 588 (2000).
[22]A. Terray, J. Oakey, and D. W. M. Marr, Science **296**, 1841 (2002).
[23]N. L. Jeon, D. T. Chiu, C. J. Wargo, H. K. Wu, I. S. Choi, J. R. Anderson, and G. M. Whitesides, Biomed. Microdevices **4**, 117 (2002).
[24]C. Yu, S. Mutlu, P. Selvaganapathy, C. H. Mastrangelo, F. Svec, and J. M. J. Frechett, Anal. Chem. **75**, 1958 (2003).
[25]W. H. Grover, R. H. C. Ivester, E. C. Jensen, and R. A. Mathies, Lab Chip **6**, 623 (2006).
[26]B. J. Kirby, D. S. Reichmuth, R. F. Renai, T. J. Shepodd, and B. J. Wiedenman, Lab Chip **5**, 184 (2005).
[27]W. Gu, X. Y. Zhu, N. Futai, B. S. Cho, and S. Takayama, Proc. Natl. Acad. Sci. U.S.A. **101**, 15861 (2004).
[28]J. Sutanto, P. J. Hesketh, and Y. H. Berthelot, J. Micromech. Microeng. **16**, 266 (2006).
[29]H.-B. Liu, E. K. Ting, and H.-Q. Gong, Biomicrofluidics **6**, 012815 (2012).
[30]D. B. Weibel, M. Kruithof, S. Potenta, S. K. Sia, A. Lee, and G. M. Whitesides, Anal. Chem. **77**, 4726 (2005).
[31]Y. Z. Zheng, W. Dai, and H. K. Wu, Lab Chip **9**, 469 (2009).
[32]Y. N. Wang, C. H. Tsai, L. M. Fu, and L. K. L. Liou, Biomicrofluidics **7**, 044118 (2013).
[33]G. Li, Y. H. Luo, Q. Chen, L. Y. Liao, and J. L. Zhao, Biomicrofluidics **6**, 014118 (2012).
[34]Z. Han, X. Tang, and B. Zheng, J. Micromech. Microeng. **17**, 1828 (2007).
[35]X. Tang and B. Zheng, Analyst **136**, 1222 (2011).
[36]G. G. Yaralioglu, I. O. Wygant, T. C. Marentis, and B. T. Khuri-Yakub, Anal. Chem. **76**, 3694 (2004).
[37]Z. Yang, S. Matsumoto, H. Goto, M. Matsumoto, and R. Maeda, Sens. Actuators A **93**, 266 (2001).
[38]D. B. Craig, E. A. Arriaga, J. C. Y. Wong, H. Lu, and N. J. Dovichi, J. Am. Chem. Soc. **118**, 5245 (1996).
[39]Y. F. Li, D. M. Guo, and B. Zheng, RSC Adv. **2**, 4857 (2012).